

APPENDIX

**APPENDIX A THE CHROMATOGRAMS AND CALIBRATION CURVE OF
METHOD 1**

Table 16 The peak areas of niacinamide at various concentrations of niacinamide standard

| Niacianmide Concentration (µg/ml) | Absorbance of peak area | | | Mean | SD |
|---|-------------------------|----------|----------|----------|-------|
| | N1 | N2 | N3 | | |
| 3.725 | 279417 | 276160 | 277789 | 277789 | 1629 |
| 6.25 | 553998 | 548664 | 551331 | 551331 | 2667 |
| 12.5 | 1154812 | 1150365 | 1152589 | 1152589 | 2224 |
| 25 | 2354641 | 2363450 | 2359046 | 2359046 | 4405 |
| 50 | 4754670 | 4713246 | 4733958 | 4733958 | 20712 |
| 100 | 9821553 | 9762559 | 9792056 | 9792056 | 29497 |
| 200 | 19983389 | 19895174 | 19939282 | 19939282 | 44108 |

Table 17 The peak areas of accuracy analysis of standard niacinamide

| Niacianmide Concentration (µg/ml) | Mean of absorbance of peak area | | |
|---|---------------------------------|--------------|---------------|
| | N1 | N2 | N3 |
| 6.25 | 382289 ± 9769 | 404209±1480 | 363790 ± 2763 |
| 12.5 | 995402±545200 | 1019974±8181 | 997935 ±1659 |
| 25 | 2340168±4277 | 317122±15915 | 2277736±5745 |

Table 18 The peak areas of precision analysis of standard niacinamide

| Niacianmide Concentration ($\mu\text{g/ml}$) | Mean of absorbance of peak area | | |
|--|---------------------------------|----------------------|----------------------|
| | N1 | N2 | N3 |
| 6.25 | 568233 \pm 14424 | 594523 \pm 17915 | 562002 \pm 15099 |
| 50 | 4344088 \pm 161163 | 4598621 \pm 140053 | 4343381 \pm 134540 |
| 100 | 8921242 \pm 376738 | 9431071 \pm 345542 | 8921554 \pm 259994 |

Table 19 The peak areas of niacinamide at various concentrations of niacinamide standard

| Niacianmide Concentration ($\mu\text{g/ml}$) | Absorbance of peak area | | | Mean | SD |
|--|-------------------------|---------|---------|---------|--------|
| | N1 | N2 | N3 | | |
| 0.0977 | 6035 | 6077 | 6028 | 6047 | 27 |
| 0.1953 | 14957 | 14213 | 14783 | 14651 | 389 |
| 0.3906 | 23097 | 23862 | 23224 | 23394 | 410 |
| 1.5625 | 113370 | 113745 | 112656 | 113257 | 553 |
| 6.25 | 485984 | 484209 | 489470 | 486554 | 2676 |
| 25 | 1795486 | 1776128 | 1782176 | 1784597 | 9903 |
| 100 | 7081203 | 7192874 | 6982012 | 7085363 | 105493 |

Table 20 The peak areas of accuracy analysis of standard niacinamide

| Niacianmide Concentration ($\mu\text{g/ml}$) | Mean of absorbance of peak area | | |
|--|---------------------------------|---------------------|----------------------|
| | N1 | N2 | N3 |
| 0.7812 | 67507 \pm 1785 | 69956 \pm 2864 | 71005 \pm 1543 |
| 12.5 | 892203 \pm 19203 | 870252 \pm 7493 | 854789 \pm 21192 |
| 100 | 7153058 \pm 162863 | 6869464 \pm 94750 | 6861253 \pm 293734 |

Table 21 The peak areas of precision analysis of niacinamide standard

| Niacianmide Concentration ($\mu\text{g/ml}$) | Mean of absorbance of peak area | | |
|--|---------------------------------|---------------------|---------------------|
| | N1 | N2 | N3 |
| 0.7812 | 33770 \pm 1577 | 35339 \pm 1417 | 34168 \pm 498 |
| 12.5 | 835269 \pm 2893 | 843794 \pm 3739 | 836007 \pm 9005 |
| 50 | 3568720 \pm 162328 | 3543183 \pm 73989 | 3635387 \pm 97403 |

APPENDIX B PARTICLES SIZE AND POLYDISPERSITY INDEX

Table 22 Size and polydispersity index of particles produced by various concentrations of calcium chloride while sericin concentration was fixed at 0.5%

| Concentration of calcium chloride (%) | | Sample | | | Mean | SD |
|---|-------------------------|--------|--------|--------|--------|-------|
| | | N1 | N2 | N3 | | |
| 2 | particle size (nm) | 250.57 | 249.07 | 255.60 | 251.75 | 3.42 |
| | polydispersity index | 0.201 | 0.165 | 0.184 | 0.183 | 0.018 |
| 4 | particle size (nm) | 248.17 | 241.33 | 243.90 | 244.47 | 3.46 |
| | polydispersity index | 0.193 | 0.167 | 0.167 | 0.180 | 0.02 |
| 6 | particle size (nm) | 251.97 | 250.57 | 247.73 | 250.09 | 2.16 |
| | polydispersity index | 0.194 | 0.247 | 0.181 | 0.207 | 0.035 |
| 8 | particle size (nm) | 256.40 | 262.33 | 260.83 | 259.85 | 3.08 |
| | polydispersity index | 0.231 | 0.193 | 0.194 | 0.206 | 0.022 |

Table 23 Size and polydispersity index of particles produced by various concentrations of calcium chloride while sericin concentration was fixed at 1%

| Concentration of calcium chloride (%) | | Sample | | | Mean | SD |
|---|-------------------------|--------|--------|--------|--------|-------|
| | | N1 | N2 | N3 | | |
| 2 | particle size (nm) | 231.40 | 228.97 | 236.93 | 232.43 | 4.08 |
| | polydispersity index | 0.163 | 0.144 | 0.157 | 0.155 | 0.010 |
| 4 | particle size (nm) | 238.77 | 236.27 | 243.63 | 239.56 | 3.74 |
| | polydispersity index | 0.152 | 0.166 | 0.154 | 0.157 | 0.008 |
| 6 | particle size (nm) | 236.17 | 247.93 | 256.50 | 246.87 | 10.21 |
| | polydispersity index | 0.206 | 0.220 | 0.177 | 0.201 | 0.022 |
| 8 | particle size (nm) | 253.30 | 248.17 | 257.73 | 253.07 | 4.78 |
| | polydispersity index | 0.194 | 0.214 | 0.168 | 0.192 | 0.023 |

Table 24 Size and polydispersity index of particles produced by various concentrations of calcium chloride while sericin concentration was fixed at 2%

| Concentration of calcium chloride (%) | | Sample | | | Mean | SD |
|--|-------------------------|--------|--------|--------|--------|-------|
| | | N1 | N2 | N3 | | |
| 2 | particle size (nm) | 222.37 | 228.43 | 228.00 | 226.27 | 3.38 |
| | polydispersity index | 0.147 | 0.146 | 0.145 | 0.146 | 0.001 |
| 4 | particle size (nm) | 251.27 | 254.57 | 256.33 | 254.06 | 2.57 |
| | polydispersity index | 0.210 | 0.182 | 0.203 | 0.198 | 0.015 |
| 6 | particle size (nm) | 261.03 | 261.77 | 263.47 | 262.09 | 1.25 |
| | polydispersity index | 0.211 | 0.247 | 0.200 | 0.219 | 0.025 |
| 8 | particle size (nm) | 250.90 | 248.90 | 235.03 | 244.94 | 8.64 |
| | polydispersity index | 0.182 | 0.218 | 0.254 | 0.218 | 0.036 |

Table 25 Size and polydispersity index of particles produced by various homogenizing speed

| Homogenizing speed (rpm) | | Sample | | | Mean | SD |
|--------------------------|----------------------|--------|--------|--------|--------|-------|
| | | N1 | N2 | N3 | | |
| 6500 | particle size (nm) | 222.37 | 228.43 | 228.00 | 226.27 | 3.38 |
| | polydispersity index | 0.147 | 0.146 | 0.145 | 0.146 | 0.001 |
| 9500 | particle size (nm) | 230.40 | 236.30 | 237.90 | 234.87 | 3.95 |
| | polydispersity index | 0.164 | 0.177 | 0.149 | 0.163 | 0.037 |
| 13500 | particle size (nm) | 215.72 | 232.56 | 224.30 | 224.19 | 8.42 |
| | polydispersity index | 0.172 | 0.198 | 0.19 | 0.187 | 0.013 |

Table 26 Size and polydispersity index of particles produced by various homogenizing time

| Homogenizing speed (rpm) | | Sample | | | Mean | SD |
|--------------------------|----------------------|--------|--------|--------|--------|-------|
| | | N1 | N2 | N3 | | |
| 15 | particle size (nm) | 249.57 | 250.78 | 235.44 | 245.26 | 8.53 |
| | polydispersity index | 0.154 | 0.164 | 0.170 | 0.162 | 0.008 |
| 30 | particle size (nm) | 238.97 | 255.23 | 252.67 | 248.96 | 8.74 |
| | polydispersity index | 0.21 | 0.228 | 0.177 | 0.205 | 0.026 |
| 60 | particle size (nm) | 222.37 | 228.43 | 228.00 | 226.27 | 3.38 |
| | polydispersity index | 0.147 | 0.146 | 0.145 | 0.146 | 0.001 |

Table 27 Size and polydispersity index of particles produced by various homogenizing time while homogenizing speed was fixed at 1000 rpm

| Homogenizing speed (rpm) | | Sample | | | Mean | SD |
|--------------------------|----------------------|--------|--------|--------|--------|-------|
| | | N1 | N2 | N3 | | |
| 15 | particle size (nm) | 256.80 | 239.87 | 255.20 | 250.62 | 9.35 |
| | polydispersity index | 0.198 | 0.176 | 0.188 | 0.187 | 0.011 |
| 30 | particle size (nm) | 269.93 | 268.57 | 260.30 | 266.27 | 5.21 |
| | polydispersity index | 0.192 | 0.204 | 0.175 | 0.190 | 0.015 |
| 60 | particle size (nm) | 254.80 | 264.70 | 274.47 | 264.66 | 9.83 |
| | polydispersity index | 0.201 | 0.153 | 0.233 | 0.196 | 0.040 |

APPENDIX C ENTRAPMENT EFFICIENCY AND NIACINAMIDE LOADING OF NIACINAMIDE LOADED SERICIN NANOPARTICLES

The calculation of % entrapment efficiency (EE)

The %entrapment efficiency was calculated by using the equation:

$$\%EE = \frac{W_{\text{particles}}}{W_{\text{ini}}} \times 100$$

Where

% EE = percentage of entrapment efficiency

$W_{\text{particles}}$ = amount of total of niacinamide in sericin nanoparticles

W_{ini} = amount of initial total of niacinamide used for the preparation

Table 28 Percentage of entrapment efficiency in various conditions

| Preparing condition | | | | | | Entrapment (%) | | | | |
|---------------------|-------------|-------------|--------------------------|-------------------|-------|----------------|-------|-------|-------|--|
| Niacinamide (%) | Acetone (%) | DC5225C (%) | Heating temperature (°C) | Heating time (hr) | N1 | N2 | N3 | Mean | SD | |
| 20 | 15 | 5 | 50 | 1 | 0.40 | 0.15 | 0.71 | 0.42 | 0.28 | |
| 20 | 15 | 5 | 50 | 5 | 1.77 | 1.47 | 1.40 | 1.55 | 0.20 | |
| 5 | 15 | 5 | 50 | 5 | 1.61 | 2.19 | 2.44 | 2.08 | 0.43 | |
| 5 | 15 | 5 | 80 | 5 | 4.13 | 4.1 | 4.04 | 4.09 | 0.048 | |
| 1 | 15 | 5 | 80 | 5 | 25.61 | 32.48 | 29.57 | 29.22 | 8.45 | |
| 0.5 | 15 | 5 | 80 | 5 | 66.69 | 55.61 | 53.77 | 58.69 | 6.99 | |
| 0.5 | 15 | 1 | 80 | 5 | 50.10 | 65.85 | 62.28 | 59.41 | 8.26 | |

The calculation of % niacinamide loading

The niacinamide loading was calculated by using the equation:

$$\% \text{ Niacinamide loading} = \frac{W_{\text{particle}}}{\text{Niac}} \times 100$$

Where

W_{particle} = amount of total of niacinamide in niacinamide-loaded sericin nanoparticles, Niac = amount of niacinamide

Table 29 Percentage of niacinamide loading at various conditions

| Preparing condition | | | | | Loading (%) | | | | |
|---------------------|-------------|-------------|--------------------------|-------------------|-------------|-------|-------|-------|-------|
| Niacinamide (%) | Acetone (%) | DC5225C (%) | Heating temperature (°C) | Heating time (hr) | N1 | N2 | N3 | Mean | SD |
| 20 | 15 | 5 | 50 | 1 | 0.144 | 0.138 | 0.11 | 0.131 | 0.018 |
| 20 | 15 | 5 | 50 | 5 | 0.187 | 0.2 | 0.172 | 0.186 | 0.014 |
| 5 | 15 | 5 | 50 | 5 | 0.392 | 0.301 | 0.336 | 0.343 | 0.046 |
| 5 | 15 | 5 | 80 | 5 | 0.458 | 0.499 | 0.502 | 0.486 | 0.025 |
| 1 | 15 | 5 | 80 | 5 | 0.411 | 0.523 | 0.521 | 0.485 | 0.064 |
| 0.5 | 15 | 5 | 80 | 5 | 0.455 | 0.463 | 0.506 | 0.475 | 0.027 |
| 0.5 | 15 | 1 | 80 | 5 | 0.512 | 0.54 | 0.461 | 0.504 | 0.040 |

**APPENDIX D STABILITY OF NIACINAMIDE IN SERICIN
NANOPARTICLES**

The calculation of percentages remaining of niacinamide

$$\% \text{ Niacinamide remaining} = \frac{CI}{CT}$$

where

CI is the amount of niacinamide initially detected in sample

CT is the amount of niacinamide at various conditions.

Table 30 Percentages remaining of niacinamide in buffer pH 5.5 at tested temperature

| Temperature | Percentages remaining of niacinamide | | | Mean | SD |
|-------------|--------------------------------------|--------|--------|-------|------|
| | N1 | N2 | N3 | | |
| 25°C | 86.00 | 85.94 | 87.97 | 86.64 | 1.16 |
| control | 96.92 | 86.63 | 87.92 | 90.49 | 5.61 |
| 50°C | 96.073 | 91.149 | 92.930 | 93.38 | 2.49 |
| control | 86.09 | 90.29 | 93.55 | 89.98 | 3.74 |

Table 31 Percentages remaining of niacinamide in buffer pH 7.4 at tested temperature

| Temperature | Percentages remaining of niacinamide | | | Mean | SD |
|-------------|--------------------------------------|-------|--------|-------|------|
| | N1 | N2 | N3 | | |
| 25°C | 93.98 | 95.63 | 101.20 | 96.94 | 3.78 |
| control | 92.25 | 93.94 | 93.62 | 93.27 | 0.90 |
| 50°C | 89.28 | 92.68 | 94.65 | 92.21 | 2.72 |
| control | 88.37 | 89.11 | 86.85 | 88.11 | 1.15 |

**APPENDIX E *IN VITRO* RELEASE OF NIACINAMIDE FROM
 NIACINAMIDE-LOADED SERICIN NANOPARTICLES IN
 PHOSPHASE BUFFER**

**Table 32 *In vitro* release of niacinamide from niacinamide-loaded sericin
 nanoparticles phosphate buffer pH 5.5**

| Time (hr) | Percentage of niacinamide releasing | | | Mean | SD |
|-----------|-------------------------------------|--------|--------|--------|------|
| | N1 | N2 | N3 | | |
| 0.25 | 42.84 | 45.78 | 44.95 | 44.52 | 1.52 |
| 0.5 | 50.20 | 49.32 | 48.75 | 49.43 | 0.73 |
| 0.75 | 97.04 | 88.79 | 85.44 | 90.42 | 5.97 |
| 1 | 95.08 | 95.42 | 94.79 | 95.10 | 0.32 |
| 2 | 100.98 | 95.32 | 97.78 | 98.03 | 2.84 |
| 4 | 101.45 | 97.44 | 102.87 | 100.59 | 2.82 |
| 6 | 97.63 | 99.30 | 97.67 | 98.20 | 0.95 |
| 8 | 101.48 | 100.99 | 100.21 | 100.89 | 0.64 |

Table 33 *In vitro* release of niacinamide from niacinamide-loaded sericin nanoparticles in phosphate buffer pH 7.4

| Time (hr) | Percentage of niacinamide releasing | | | Mean | SD |
|-----------|-------------------------------------|--------|--------|--------|-------|
| | N1 | N2 | N3 | | |
| 0.25 | 57.03 | 67.54 | 68.99 | 64.52 | 6.53 |
| 0.5 | 59.06 | 77.32 | 59.36 | 65.25 | 10.46 |
| 0.75 | 100.59 | 91.86 | 89.11 | 93.85 | 6.00 |
| 1 | 95.25 | 96.97 | 98.07 | 96.77 | 1.42 |
| 2 | 94.26 | 104.70 | 86.44 | 101.57 | 9.16 |
| 4 | 88.17 | 96.90 | 92.68 | 96.86 | 4.36 |
| 6 | 99.71 | 94.31 | 100.03 | 97.45 | 3.22 |
| 8 | 102.47 | 103.96 | 101.05 | 102.49 | 1.46 |

APPENDIX F SKIN PERMIATION STUDY

Table 34 Percentage of niacinamide permeated from niacinamide-loaded sericin nanoparticles lotion

| Time (hr) | Percentages of cumulative amount of niacinamide | | | | | Mean | SE |
|--------------------|---|-------|-------|-------|-------|-------|------|
| | N1 | N2 | N3 | N4 | N5 | | |
| 1 | 1.98 | 2.29 | 2.24 | 1.56 | 2.07 | 2.03 | 0.13 |
| 2 | 2.46 | 3.10 | 1.97 | 2.69 | 1.92 | 2.43 | 0.22 |
| 4 | 2.48 | 2.91 | 2.48 | 2.59 | 2.89 | 2.67 | 0.10 |
| 6 | 2.18 | 3.32 | 2.79 | 2.12 | 3.91 | 2.86 | 0.34 |
| 8 | 2.34 | 3.25 | 2.36 | 4.56 | 4.44 | 3.39 | 0.47 |
| donor | 45.17 | 55.26 | 51.42 | 52.28 | 50.70 | 50.96 | 1.64 |
| total strips | 22.16 | 25.89 | 26.50 | 20.98 | 17.47 | 22.60 | 1.66 |
| living skin strata | 25.75 | 19.63 | 17.41 | 14.80 | 18.41 | 19.20 | 1.82 |
| receiver | 2.34 | 3.25 | 2.36 | 4.56 | 4.44 | 3.39 | 0.48 |

Table 35 Percentage of niacinamide permeated from niacinamide lotion

| Time (hr) | Percentages of cumulative amount of niacinamide | | | | | Mean | SE |
|--------------------------|---|-------|----------------|-------|-------|-------|------|
| | N1 | N2 | N3 | N4 | N5 | | |
| 1 | | | | | | | |
| 2 | | | | | | | |
| 4 | | | | | | | |
| 6 | | | Lower than LOQ | | | | |
| 8 | | | | | | | |
| donor | 63.10 | 69.90 | 53.05 | 57.43 | 55.54 | 59.80 | 2.86 |
| total strips | 12.80 | 14.65 | 12.95 | 11.40 | 12.97 | 12.95 | 0.49 |
| living skin strata | 16.97 | 14.26 | 19.96 | 7.98 | 15.29 | 14.89 | 1.88 |